Locally administered immunotherapy self-delivering RNAi PH-762 results in abscopal clearance of untreated distal tumors, suggesting systemic immune response, in a murine hepatocarcinoma model

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Abstract

Background Local administration of immune checkpoint inhibition (ICI) holds potential toward providing enhanced activity and decreased systemic toxicity, but such an approach is challenging with the available ICI antibodies. We have previously shown that local delivery of PH-762, a self-delivering RNAi compound targeting PD-1 based on proprietary INTASYL™ technology, produces robust PD-1 silencing and associated anti-tumor immunophenotypic changes in the tumor microenvironment. Here, we present data indicating that local administration of PH-762 not only inhibits local tumor growth but can also elicit an abscopal effect in distal untreated tumors. The *in vivo* efficacy and *in vitro* mechanism of action support the generation of a PH-762 driven systemic anti-tumor immune response. Therefore, ICI using INTASYL is an alternative to antibody drugs for immunotherapy.

Methods To assess *in vivo* efficacy, Hepa1-6 cells were implanted subcutaneously into the flanks of C57BL/6J mice (N = 5 / group). Vehicle (PBS) or murine targeting PH-762 (mPH-762) were administered intratumorally (IT) on Days 1, 4, 7, 10 and 14. To determine an abscopal effect cells were also implanted into the opposite flank but left untreated. Treatment was initiated when primary tumors reached a mean volume of 150 mm^3. Tumor volumes and body weights were recorded longitudinally. In addition, *in vitro* mechanism of action studies were performed with CD3-stimulated human pan T cells. PD-1 mRNA knockdown was assessed by qRT-PCR; PD-1 protein expression by flow cytometry; and T cell function by cytokine release.

Results Treatment with IT administered mPH-762 significantly inhibited tumor growth compared with vehicle treated control tumors. Furthermore, the growth of the untreated bilateral tumor was significantly reduced with 80% of these tumors showing complete regression. Mechanism of action studies showed potent and durable silencing of PD-1. Increased release of IFN-γ, CXCL10, and IL-6 and suppression of IL-10 release were indicators of an enhanced immune response.

Conclusions These data show that silencing PD-1 with local administration of mPH-762 not only inhibits growth of treated tumors but elicits an abscopal effect leading to cure of distal tumors. This data and other recently published data showing evidence of a specific antitumor immune response in a tumor rechallenge model after prior treatment with INTASYL compounds, demonstrate the desired systemic immune response can be obtained with local administration of PH-762. INTASYL represents an alternative to antibody checkpoint therapy with potential for improved efficacy and reduced systemic toxicity which will be investigated in an upcoming clinical trial.

PH-762 potently silences PD-1 in human pan T cells

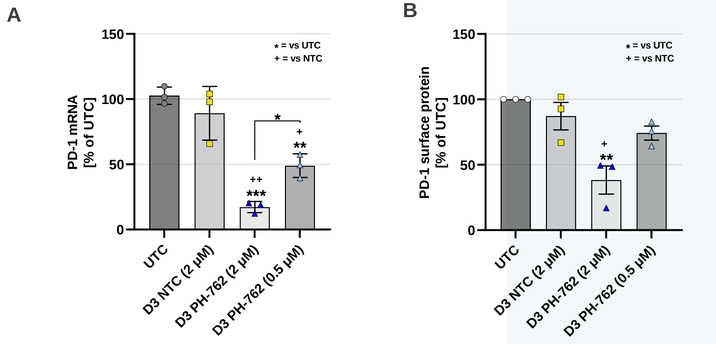
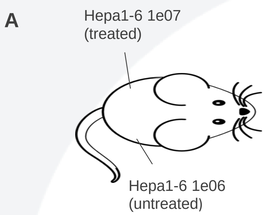


Figure 1. PH-762 potently silences PD-1 mRNA and protein in human pan T cells

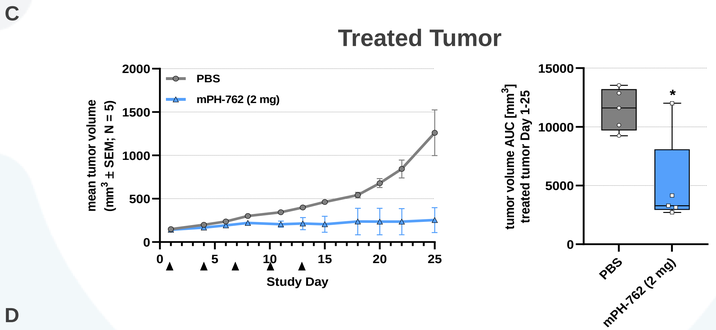
On-target silencing mediated by PH-762 in human pan T cells isolated from three individual donors. T cells were stimulated with CD3 / CD28 beads to induce PD-1 expression and treated with PH-762 or a chemically-identical but nontargeting control INTASYL (NTC). **A.** mRNA levels assessed by qRT-PCR shown as a percentage (%) relative to the untreated control (UTC) ± SEM. **B.** %PD-1-positive T cells shown as % relative to UTC. Groups were intercompared one way ANOVA and Tukey's multiple comparisons *post-hoc* tests. ***p < 0.001, **p < 0.01, *p <0.05. * = vs UTC, + = vs NTC.

Local INTASYL therapy with mPH-762 induces abscopal anti-tumor response with clearance of untreated distal tumors



B

Group	N	treated tumor inoculum (#cells)	untreated tumor inoculum (#cells)	Treatment	Dose (mg)	ROA	Schedule (Day)
1	5	1e07	1e06	PBS	0 mg	IT	1, 4, 7, 10, 13
2	5	1e07	1e06	mPH-762	2 mg	IT	



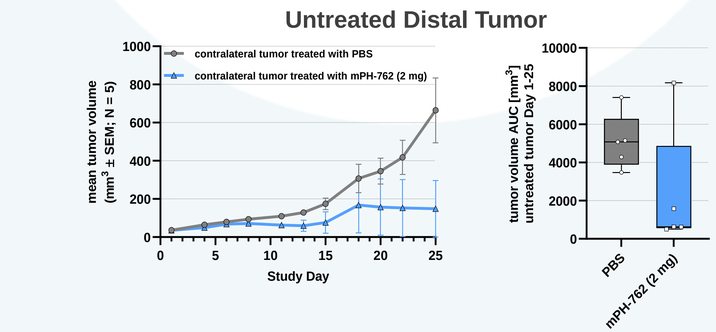
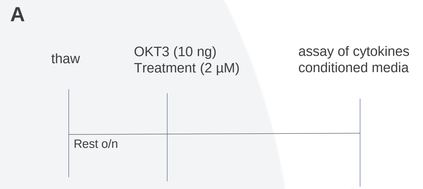


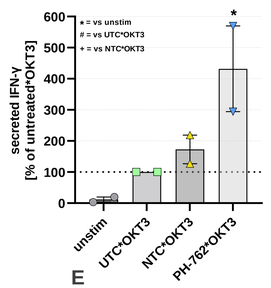
Figure 2. Intratumoral mPH-762 provides robust anti-tumor efficacy to both locally treated and untreated distal tumors

A. Schematic of seeding and treatment of Hepa1-6 tumors. **B.** Study details overview table. **C**. Efficacy of mPH-762 toward the treated tumor. **Left:** Mean tumor volume over time (mm^3 ± SEM; N = 5). **Right:** Cumulative mean tumor volume area under the curve (AUC) as calculated by trapezoidal transformation for each animal. Box and whisker plots are shown with medians indicated. Groups were compared by unpaired two-tailed student's t test. mPH-762 therapy provided statistically significant tumor control. **D** . Efficacy of mPH-762 toward the untreated distal tumor. **Left:** Mean tumor volume over time (mm^3 ± SEM; N = 5). **Right:** Cumulative mean tumor volume AUC as calculated by trapezoidal transformation for each animal. Box and whisker plots are shown with medians are indicated.

in vivo studies were performed at Pharma Models LLC, Marlborough, MA

INTASYL PH-762 enhances cytotoxic T cell function by modulating secretion of cytokines associated with antitumor immune response





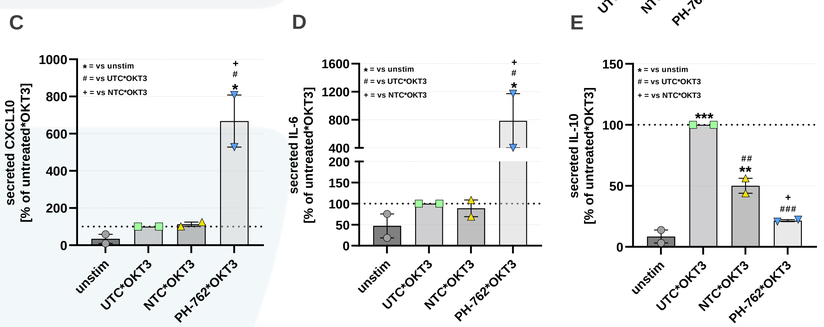


Figure 3. PH-762 promotes secretion of cytokines / chemokines associated with immune response and reduces immunosuppressive IL-10 in primary human pan T cells

A. Experiment design schematic. Human pan T cells from two individual donors were stimulated with 10 ng plate-bound OKT3 overnight to induce PD-1 expression and treated with PH-762 or a chemically identical, but non-targeting control INTASYL (NTC). **B-E.** Conditioned media was collected from the individual T cell cultures on Day 3 post stim / treatment and cytokine levels assessed by multiplex cytometric bead array assay). Cytokines secreted from pan T cells derived from two individual human donors shown as percentage relative to the OKT3 stimulated untreated condition. **C.** IFN-γ. **D.** CXCL10. **E.** IL-6. **F.** IL-10. Conditions were intercompared by one way ANOVA and Tukey's multiple comparisons *post-hoc* test p < 0.05, **p < 0.01, ***p < 0.001. * = vs unstimulated/untreated (unstim); # = vs OKT3 stimulated/untreated (untreated*OKT3); + = vs OKT3 stimulated / treated with NTC (NTC*OKT3).

Conclusions

- **Local treatment with mPH-762 provides robust anti-tumor efficacy to both locally treated tumors and to untreated distal tumors, suggesting an abscopal effect, due to a systemic immune response.**
- **PH-762 provides potent silencing of PD-1 in human T cells.**
- **PH-762 promotes secretion of cytokines and chemokines associated with adaptive antitumor immune response and concomitant decrease in immunosuppressive IL-10 in human pan T cells.**
- **The systemic anti-tumor immune response toward distant tumors and metastases following local administration of PH-762 will be investigated in an upcoming Phase 1b clinical trial.**